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(54) ANTIMICROBIAL MULTIPLE ANTIGEN PEPTIDE

(57)Abstract:
PROBLEM TO BE SOLVED: To obtain the subject new peptide, containing an antimicrobially active moiety of lactoferrin and useful as a cationic antimicrobial agent or the like by connecting the N-terminals of a branched type peptide containing a specific number of connected lysines to the C-terminal of a peptide having a specific amino acid sequence.
SOLUTION: This new antimicrobial multiple antigen peptide is obtained by respectively connecting the 16 N-terminals of a branched type peptide containing 15 connected lysines (Lys) to the C-terminal of a peptide composed of an amino acid represented by the formula and is useful as an active ingredient or the like of a medical preparation having antimicrobial activities against Escherichia coli, Staphylococcus aureus, fungi or the like. The antimicrobial multiple antigen peptide is obtained by dissolving lysine in aqueous dioxane, adding and reacting a 1MNaOH and (Boc)2O (Boc is t-butyloxycarbonyl) with the resultant solution under cooling with ice, then acidifying the reactional solution, extracting the acidified reactional solution with ethyl acetate, vacuum concentrating the extract, carrying out the crystallization, producing a branched type peptide containing 15 connected lysines, subsequently deprotecting the resultant peptide and then binding the N-terminals of the deprotected peptide to the C-terminal of the peptide represented by formula.

Phe Gln Trp Gln Arg Asn Met Arg Lys Val Arg

LEGAL STATUS

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[Date of extinction of right]	

* NOTICES *

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CLAIMS

[Claim(s)]
[Claim 1] The antibacterial multiple antigen peptide which comes to connect the C terminal of the peptide which becomes 16 amino terminals of the branch-type peptide which 15 lysines (Lys) connected from the following amino acid sequence, respectively.
Phe Gln Trp Gln Arg Asn Met Arg Lys Val Arg -- [Claim 2] The cation nature antimicrobial agent which contains an antibacterial multiple antigen peptide according to claim 1 as an active principle.
[Claim 3] The cation nature antimicrobial agent according to claim 2 whose concentration of an active principle is more than 2microM.

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DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] This invention relates to the cation nature antimicrobial agent which makes an active principle an antibacterial multiple antigen peptide and this in detail about the antibacterial peptide for medical pharmaceutical preparation which has antibacterial [over Escherichia coli, Staphylococcus aureus, or a fungus].
[0002]
[Description of the Prior Art] Lactoferrin is the huge protein of molecular weight 80,000 [about] (703 amino acid), and is protein in which the antimicrobial activity contained in mother's milk, tear fluid, etc. is shown. The application of the remedy of the various symptoms accompanying infectious disease prevention or an immunity force fall, trauma medicine, and eye drops is expected, and Homo sapiens lactoferrin is used as an additive for powdered milk for sucklings.
[0003] By the way, the Europe biochemistry meeting magazine (FEBS:Federation of European Biochemical Societies), To No. 382 and the 175-178th page in 1996 Lactoferrin 46 base, its 20 to hole loop-formation 35 base (HLT1), and short peptide The antibacterial action of 24 to Phe Gln Trp Gln Arg Asn Met Arg Lys Val Arg 35 base (HLT2) The research result of Edward and others compared using the Escherichia coli strain (strains) 8007 and ML35 is indicated.
[0004] According to it, although antibacterial is hardly accepted in lactoferrin itself, with the short peptides HLT1 or HLT2, there is antibacterial effectiveness of decreasing Escherichia coli 107 CFU of control/ml (CFU showing colony unit:colony forming unit.) to 300 CFU/ml by about 300microM, namely, HTL1 or HTL2 show a stronger antibacterial action from the whole lactoferrin. Moreover, it is also indicated that HLT1 which 16 amino acid combined shows an antibacterial action stronger than short HLT2 5 residue from this.
[0005] Moreover, to JP,5-92994,A, lactoferrin is formed into a short peptide using an acid or an enzyme, and the result of having examined the antibacterial action using 26 sorts of gram negative stocks, 14 sorts of gram positive stocks, and two sorts of yeast is indicated.
[0006]
[Problem(s) to be Solved by the Invention] however -- according to above-mentioned JP,5-92994,A -- Aspergillus Fumi Gay -- JCM of TASS (Aspergillus fumigatus) 1739I share and RIZOPASU ORIZAE (Rizopus oryzae) Resistance was shown to the short peptide of the lactoferrin origin, and this short peptide did not demonstrate antibacterial [sufficient]. Moreover, there is no example which used HLT2 with weak antimicrobial activity positively compared with HLT1.
[0007] Then, the technical problem of this invention is offering the peptide which demonstrates antibacterial [which was conventionally excellent compared with elegance] using the specific short peptide with which an antibacterial action's is accepted among the amino acid sequences which constitute lactoferrin, and offering the antimicrobial agent which has still such antibacterial.
[0008]
[Means for Solving the Problem] In order to solve the above-mentioned technical problem, in this

invention, it considered as the antibacterial multiple antigen peptide which comes to connect the C terminal of the peptide which becomes 16 amino terminals of the branch-type peptide which 15 lysines (Lys) connected from the following amino acid sequence, respectively.
It considered as the cation nature antimicrobial agent which considered as Phe Gln Trp Gln Arg Asn Met Arg Lys Val Arg or the cation nature antimicrobial agent which contains the above-mentioned antibacterial multiple antigen peptide as an active principle, and carried out the active principle concentration (mol concentration) to more than 2microM preferably.
[0009] The antibacterial multiple antigen peptide of a configuration of having described above connects the short peptide of said predetermined amino acid sequence with 16 amino terminals of the branch-type peptide which 15 lysines (Lys) connected, and demonstrates antibacterial [which was not able to be demonstrated only with the short peptide of said predetermined amino acid sequence / outstanding] (the below-mentioned example shows.).
[0010] Moreover, the above-mentioned antibacterial multiple antigen peptide is a cation nature peptide, and the antibacterial action is an operation which unlike bacteria growth inhibition, i.e., DNA synthesis inhibition, and synthetic enzyme inhibition the membrane potential of a bacterial plaque is made to start a variation rate, and destroys the film. Therefore, the above-mentioned antibacterial multiple antigen peptide cannot give resistance easily to bacteria.
[0011] Moreover, although the usual antibacterial peptide is extracted from the fungus etc. and a side effect appears strongly in the body, it is thought that it is harmless to the body and does not have a side effect since lactoferrin is a living body origin component.
[0012]
[Embodiment of the Invention] The peptide which consists of a predetermined amino acid sequence in this invention can be manufactured using the well-known peptide preparation approaches, such as a chemosynthesis method. Specifically, it can manufacture by technique, such as chemosynthesis and transgenics.
[0013] When compounding a peptide with a solid phase synthesis method, the amino acid of the C terminal of the peptide which should be compounded on insoluble base materials, such as bridge formation polystyrene, is fixed, the commercial synthesizer unit controlled by the microcomputer with it as the starting point removes a protective group suitably to N-alpha-t-butoxycarbonyl-ized amino acid (Boc-amino acid) or N-alpha-9-fluorenyl methoxycarbonyl-ized amino acid (Fmoc-amino acid), and order is made to elongate a necessary peptide chain. Incidentally, as the manufacturing company or selling firm of a peptide synthesis machine, he is Pharmacia. There are a biotechnology theque company, PerkinElmer Japan Co., Ltd., Aloka, Shimadzu make, etc.
[0014] MAP of the 16 time object of a short peptide (Multiple antigen peptide) In order to manufacture, Boc-Lys (Boc) or Fmoc-Lys (Fmoc) is used for insoluble base materials, such as bridge formation polystyrene, a branching peptide is compounded, the amino acid of the C terminal of the peptide used as an antigen is fixed there, and the approach of growing up a peptide with it as the starting point can be adopted. Boc is the third butyloxy carbonyl group and Fmoc is a fluorenyl methoxycarbonyl group.
[0015] namely, as a synthetic example of bodily MAP, 16 times A lysine is melted in dioxane water, one-mol NaOH and 2 (Boc) O are added, mixing under ice-cooling, vacuum concentration is carried out, mixing about 30 minutes at a room temperature, and then it is 5%KHSO4. It is made acidity (pH2.3). Na2 SO4 after extracting by Et OAc and rinsing all Et OAc It is made to dry, and vacuum concentration is carried out further, it crystalizes, and the example which compounds the branch-type peptide which 15 lysines (Lys) connected is given.
[0016] Boc which is the approach of compounding a peptide with the usual solid phase synthesis method in order to grow up a peptide -- law and Fmoc -- law is employable.

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DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] The explanatory view of the synthetic process of a branch-type peptide (core of MAP16)
[Drawing 2] The explanatory view showing the structure of the branch-type peptide which 15 lysines (Lys) connected
[Drawing 3] The explanatory view showing the amino acid sequence of the example 1 which consists of a branched peptide (MAP)
[Drawing 4] The graph showing the measurement chart of HPLC of an example 1
[Drawing 5] The graph showing the measurement chart of HPLC of the example 1 of a comparison

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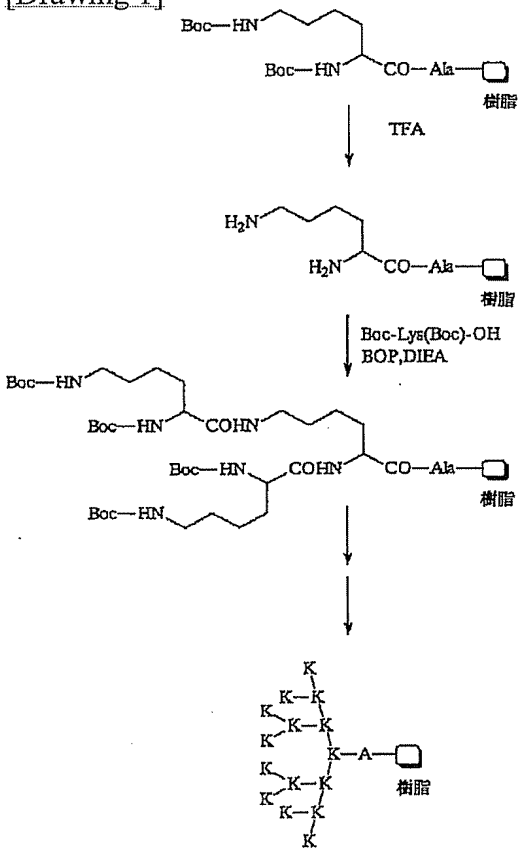
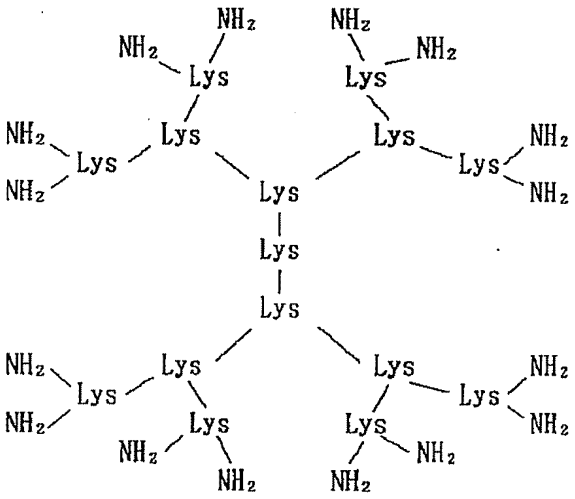
DRAWINGS

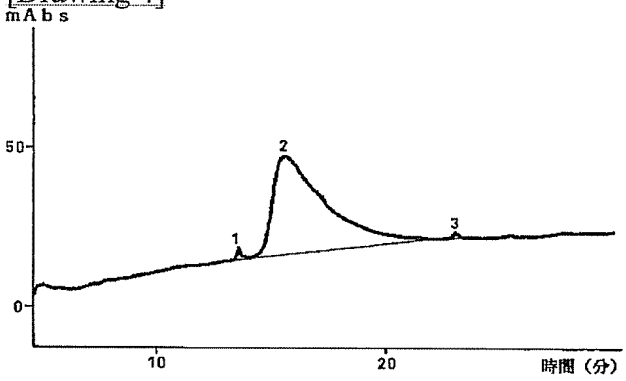
[Drawing 1]



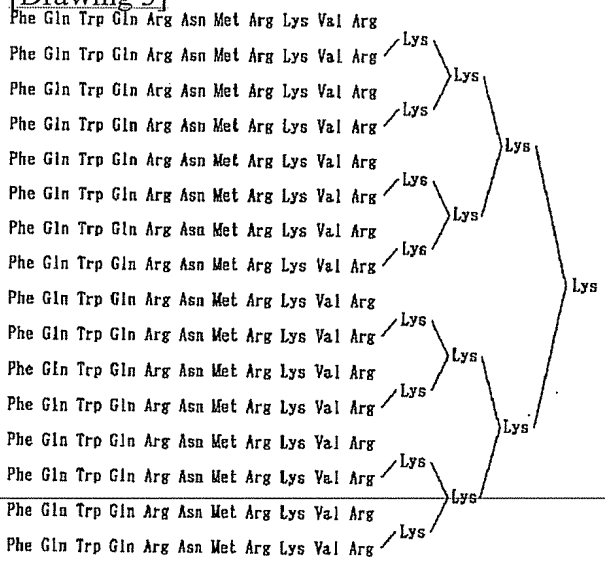
[Drawing 2]



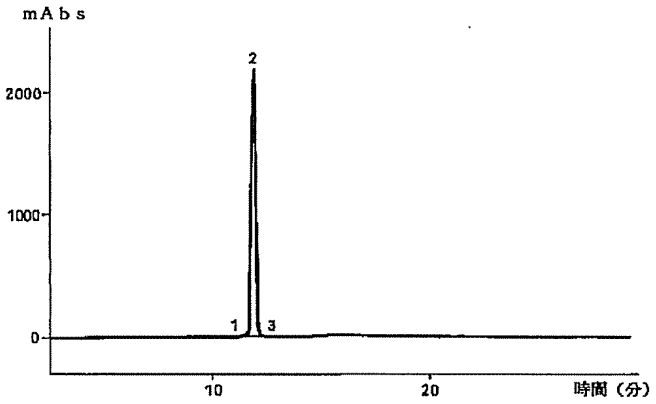
[Drawing 4]



[Drawing 3]



[Drawing 5]



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